Characteristics of an active ingredient concentrate on mitochondrial metabolism

Proof-of-principle-Testing

Since the first round of tests with the finished drink turned out negative, the following tests with the basic active ingredient should initially only serve as a "proof of principle". The multi-fruit base material yellow without sea salt (220 kg per 1000 L), sample No. 7.92731, delivery No. 81857047 of December 11, 2014 was used. EDTA blood was selected from a volunteer donor who had no chronic disease. The EDTA blood was aliquoted and incubated for 4 hours at 37 ° C. Either no substance (control), the undiluted substance or the substance in various predilutions (1:10, 1: 100, 1: 1000, 1: 100000) were added to the aliquots (Table 1). After the incubation time of 4 hours, the PBMC from each aliquot were isolated and analyzed for selected mitochondrial parameters.
The mitochondrial parameters were the ATP content, the expression of PGC-1-alpha as an indicator for mitochondrial biogenesis and the mtDNA: ntDNA ratio.

ATP-Generation

The results clearly show that regardless of the concentration used, ATP was generated. The ranges between 10% and 20% after an exposure time of 4 hours.

Mitochondrial Biogenesis (PGC-1-alpha)

The results clearly show that the active ingredient concentrate leads to an increase in PGC-1-alpha expression.

The influence of the active ingredient concentrate on PGC 1-alpha expression is concentration -dependent. The active ingredient concentrate is able to increase the PGC-1-alpha expression from 100% to> 400%.

Mitochondrial mass ratio (mtDNA: ntDNA)

In addition, the ratio of mitochondrial DNA to nuclear DNA (mtDNA: ntDNA) was determined as a parameter for the mitochondrial mass. A change in the mitochondrial mass was not expected given the short exposure time of the substances to the immune cells. The determination of the mtDNA: ntDNA ratio served as a control parameter for the vitality of the peripheral blood leukocytes (PBMC). The results show that the mitochondrial mass does not change within the short exposure time of 4 hours. The values are within the normal measurement fluctuations (Figure 3).

Summary

The proof-of-principle for a pro-mitochondrial effect of the active ingredient concentrate has been successful. The tested parameters ATP generation and PGC-1-alpha expression are suitable as indicators for the analysis of mitochondrial function. Since the pro-mitochondrial effect of the active ingredient concentrate also depends on the condition of the consumer's mitochondria, further investigations should first be carried out in vitro with different users.

Deployed-Pre-dilution of the active ingredient base substance	Active ingredient matrix on 4 ml of blood (from 1.2 to 1.5 x 10^7 PBMC)	Active ingredient basic substance to 1 ml of blood	Active ingredient basic substance to 5 l of blood	Volume drink on 5 liters of blood
undiluted	100 mg	25 mg	125.000 mg	500 ml
1:10	10 mg	2,5 mg	12.500 mg	50 ml
1:100	1 mg	0,25 mg	1250 mg	5 ml
1:1000	0,1 mg	0,025 mg	125 mg	0,5 ml
1:10000	0,01 mg	0,0025 mg	12,5 mg	0,05 ml
1:100000	0,001 mg	0,00025 mg	1,25 mg	0,005 ml

Table 1: Concentrations of active ingredient base substance used

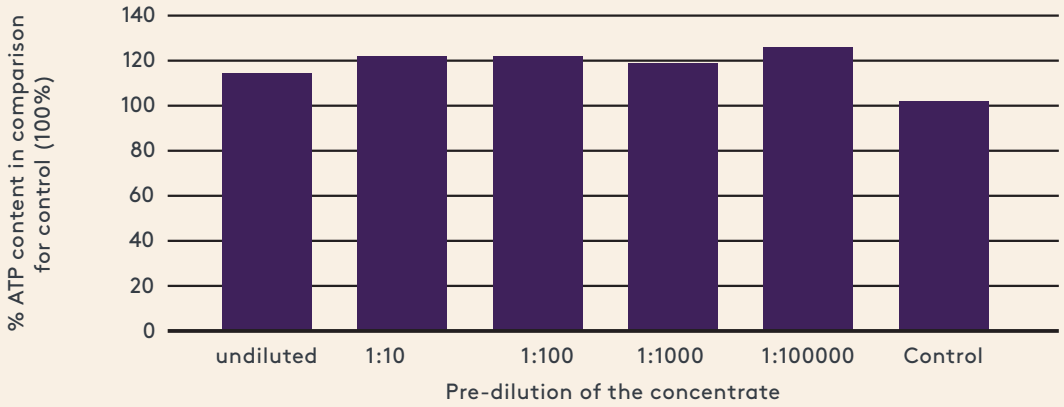

Figure 1: Influence of the active ingredient concentrate on the mitochondrial ATP content. EDTA blood was incubated for 4 hours without (control) or with the active ingredient concentrate at 37 ° C. The active ingredient concentrate was used both undiluted and diluted (predilution 1: 100, 1: 10,000, 1: 100,000). The PBMC were then isolated and the ATP content determined. The ATP content of the control stimulation was set equal to 100%

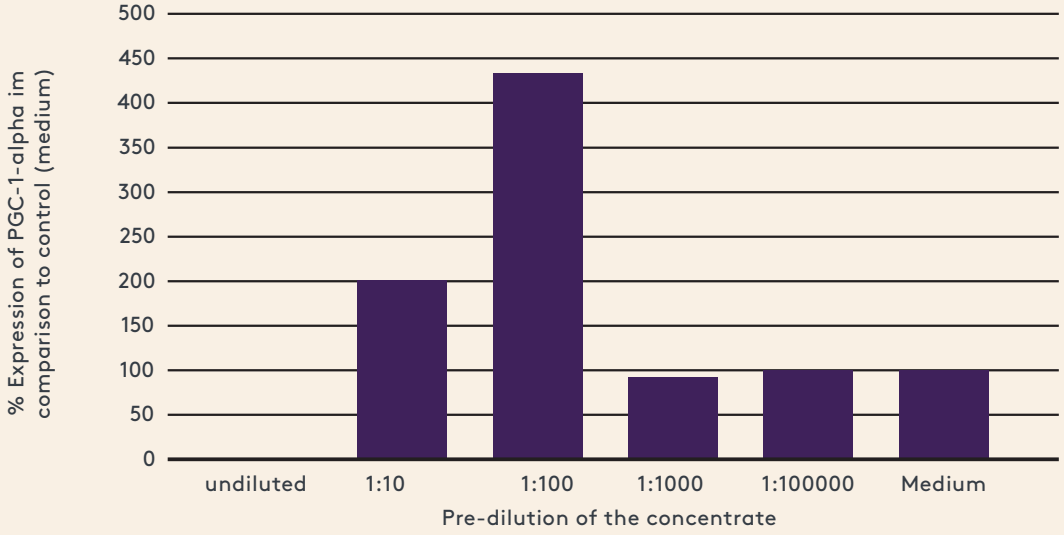

Figure 2: Influence of the active ingredient concentrate on PGC-1-alpha expression in human PBMC. EDTA blood was incubated for 4 hours without (control) or with the active ingredient concentrate at 37 ° C. The active ingredient concentrate was used both undiluted and diluted (predilution 1: 100, 1: 10,000, 1: 100,000). The PBMC were then isolated and the PGC-1-alpha expression determined. The PGC-1-alpha expression of the control stimulation was set equal to 100%.

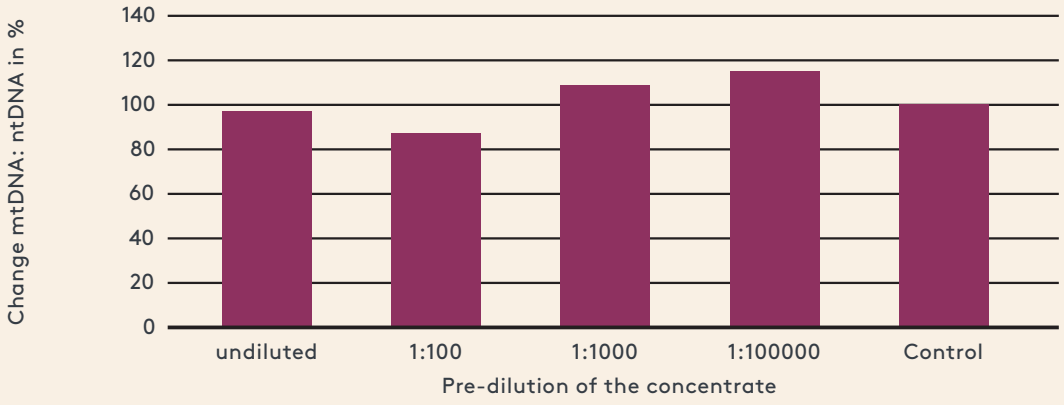

Figure 3: Influence of the active ingredient concentrate on the mtDNA: ntDNA ratio. EDTA blood was incubated for 4 hours without (control) or with the active ingredient concentrate at 37 ° C. The active ingredient concentrate was used both undiluted and diluted (predilution 1: 100, 1: 10,000, 1: 100,000). The PBMC were then isolated and the mtDNA: ntDNA ratio was determined. The ratio mtDNA: ntDNA of the control stimulation was set equal to 100%.